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Increased Toxicity of Ammonia to Rainbow Trout 'Salmo gairdneri' Resulting from Reduced Concentrations of Dissolved Oxygen

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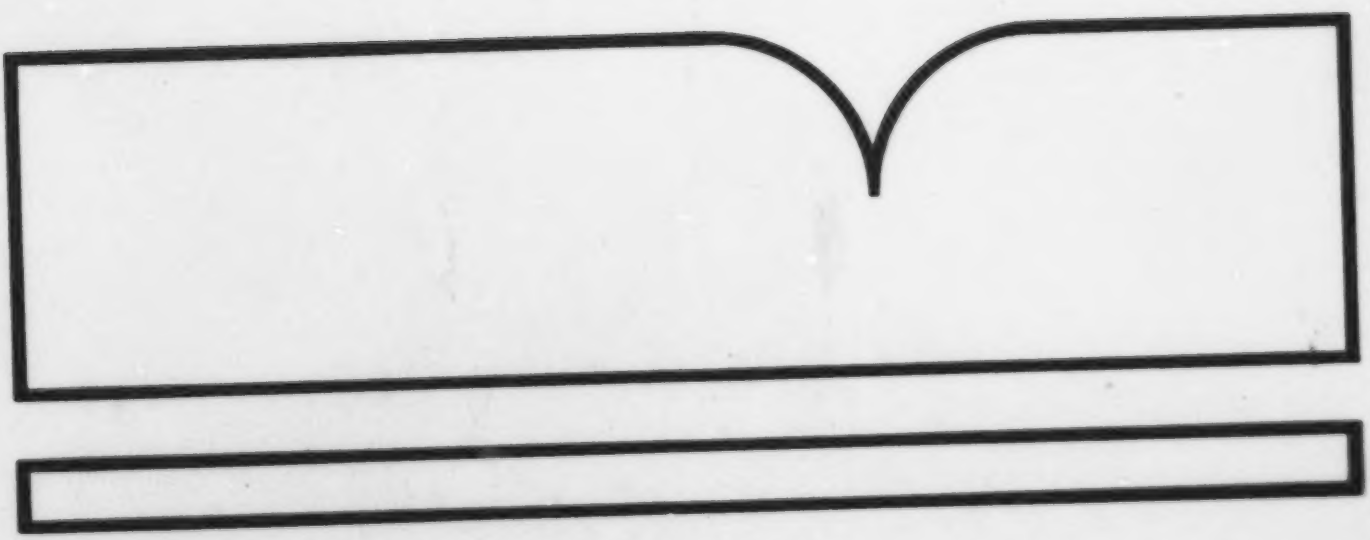

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16. ABSTRACT

The median lethal concentration (LC50) of aqueous ammonia at reduced dissolved oxygen (D.O.) concentrations was tested in acute toxicity tests with rainbow trout (Salmo gairdneri) fingerlings. Fifteen 96-h flow-through tests were conducted over the D.O. range 2.6-8.6 mg/L, the former concentration being the lowest at which control fish survived. There was a positive linear correlation between LC50 (milligrams per litre un-ionized ammonia) and D.O. over the entire D.O. range tested; ammonia toxicity increased as D.O. decreased. Ammonia LC50 values were also computed for 12, 24, 48, and 72 h; the correlation with D.O. was greater the shorter the time period.

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Increased Toxicity of Ammonia to Rainbow Trout (*Salmo gairdneri*) Resulting from Reduced Concentrations of Dissolved Oxygen

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The median lethal concentration (LC50) of aqueous ammonia at reduced dissolved oxygen (D.O.) concentrations was tested in acute toxicity tests with rainbow trout (*Salmo gairdneri*) fingerlings. Fifteen 96-h flow-through tests were conducted over the D.O. range 2.6–8.6 mg/L, the former concentration being the lowest at which control fish survived. There was a positive linear correlation between LC50 (milligrams per litre un-ionized ammonia) and D.O. over the entire D.O. range tested; ammonia toxicity increased as D.O. decreased. Ammonia LC50 values were also computed for 12, 24, 48, and 72 h; the correlation with D.O. was greater the shorter the time period.

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Nous avons déterminé, au cours d'essais de toxicité aiguë avec fingerlings de truite arc-en-ciel (*Salmo gairdneri*), la concentration létale médiane (CL50) de l'ammoniac en solution aqueuse à des concentrations réduites d'oxygène dissous (O.D.). Quinze essais de 96 h à débit continu ont été effectués à des concentrations de O.D. variant de 2,6 à 8,6 mg/L, la première étant la plus faible à laquelle les poissons témoins purent survivre. Il y a corrélation linéaire positive entre la CL50 (mg/L d'ammoniac non ionisé) et O.D. sur toute la gamme de ce dernier testée; la toxicité de l'ammoniac augmente à mesure que O.D. diminue. Nous avons également calculé la CL50 de l'ammoniac après 12, 24, 48 et 72 h; plus la période est courte et plus forte est la corrélation avec O.D.

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AMMONIA is a common component in the discharges from sewage treatment plants and many industrial processes. Reduced dissolved oxygen (D.O.) concentrations are often associated with ammonia effluents because of other characteristics of ammonia-containing wastes, e.g. high chemical and biochemical oxygen demands and elevated temperature. The interactive effect of ammonia and D.O. is, therefore, an important consideration with respect to the toxicity of ammonia to aquatic life.

Several researchers have reported that reduced D.O. concentration increases the toxicity of ammonia to fishes (Wuhrmann 1952; Wuhrmann and Woker 1953; Allan 1955; Downing and Merkens 1955; Herbert 1956; Merkens and Downing 1957; Lloyd 1961; Danecker 1964; Vámos and Tasnádi 1967; Seleši and Vamoš 1976; Alabaster et al. 1979) Results reported are that a decrease in D.O. concentration (generally 30–50% below saturation) increases the toxicity of ammonia, that at a given ammonia concentration the degree of response is correlated to D.O. concentration, that rainbow trout (*Salmo gairdneri*) are more sensitive to the combined effect of reduced D.O. and ammonia than were the other (nonsalmonid) fishes tested, and that salinity reduces the acute toxicity of ammonia to rainbow trout under reduced D.O. conditions. It has also been reported that the effect of oxygen in increasing the survival time of rainbow trout fingerlings is greater at low concentrations of ammonia than at high (Downing and Merkens 1955; Merkens and Downing 1957).

In the present study we conducted a series of fifteen 96-h flow-through toxicity tests to examine the effects of reduced D.O. concentrations on the acute toxicity of ammonia to rainbow trout fingerlings. Ammonia toxicity was measured at different concentrations of D.O. over the range 2.6–8.6 mg/L, and at different time periods. Such an approach has not been used in previously reported studies. The data were analyzed to examine (1) the effect of different concentrations of D.O. on the acute toxicity of ammonia, (2) whether such an effect varied with time, and (3) whether such an effect was greater at low than at high ammonia concentrations.

Methods — Test fish were Shasta–Manchester hybrid rainbow trout from a single lot of fish obtained from the Bozeman (Montana) Fish Cultural Development Center (FCDC), U.S. Fish & Wildlife Service (F&WS). The tests were conducted over a 2-mo period when the fish were 4 to 6 mo old. Prior to testing, the fish were reared in water (Table 1) from a ground spring located at FCDC; this same water was used for the test dilution water. During rearing, the fish were fed a F&WS hatchery production diet (Rangen Inc., Buhl, ID); they were not fed 3 d prior to, or during, test. Two tests were conducted at the normal D.O. concentration of the ground spring water and 13 tests were conducted at reduced D.O. concentrations. Reduced D.O. concentrations were achieved by mixing water taken directly from the ground spring source with water from the ground spring which had been passed through a degassing system similar to that described by Mount (1964).

Six tanks (water volume 60 L each) were used for each test, five test tanks and one control tank. Test water was delivered to the tanks by either of two methods. In the two tests at the highest D.O. concentration (8.6 mg/L), toxicant (reagent grade ammonium chloride) solution was delivered to the tanks at a flow rate of 500 mL at 2- to 3-min intervals from proportional diluters having the basic design of Mount and Brungs (1967); water replacement time in the tanks was ~5 h and full test toxicant concentration in each tank was effectively reached within 18 h. In tests at reduced oxygen concentrations, deoxygenated dilution water was delivered into the tanks

TABLE 1. Chemical characteristics of the dilution water used in bioassays (all values are milligrams per litre unless noted otherwise).

Al	<1	Ni	<0.005
As	0.0012	Pb	<0.015
Ca	52	Se	0.00085
Cd	<0.005	Zn	0.01
Cr	<0.005	Cl^-	0.16
Cu	0.007	F^-	0.35
Fe	0.004	PO_4^{3-}	0.05
Hg	<0.00005	SO_4^{2-}	17
K	0.82	Total organic carbon	3.3
Mg	17	SEC, µS 25°C	340
Mn	0.002	Turbidity, NTU	1.6
Na	2.5		

TABLE 2. Toxicity of ammonia to rainbow trout under different conditions of dissolved oxygen[a] (10 fish per tank in all tests).

Test No.	Mean fish size		D.O. (mg/L) (range)	pH (range)	Temp. (°C) (range)	96-h LC50 (95% C.I.)	
	Wt (g)	Length (cm)				mg NH_3–N/L	mg NH_3/L
397	1.7	5.7	8.61 (8.40–8.95)	7.79 (7.75–7.85)	12.4 (12.1–12.7)	42.0 —[b]	0.696 —
404	2.3	6.1	8.58 (8.40–8.90)	7.80 (7.77–7.87)	12.4 (11.8–13.1)	47.9 (43.3–53.0)	0.812 (0.734–0.899)
436	5.7	8.4	7.7 (7.5–7.9)	7.80 (7.76–7.86)	13.3 (12.9–13.8)	42.0 (37.9–46.6)	0.763 (0.689–0.846)
444	4.0	7.2	7.57 (7.40–7.75)	7.83 (7.76–7.96)	12.8 (12.6–13.0)	36.5 (31.2–42.7)	0.683 (0.584–0.800)
437	5.7	8.0	7.37 (7.10–7.65)	7.79 (7.75–7.85)	12.9 (12.7–13.2)	40.9 (36.9–45.3)	0.704 (0.636–0.780)
430	5.7	8.0	6.60 (6.50–6.90)	7.75 (7.70–7.82)	12.5 (12.3–12.7)	37.0 (33.4–40.9)	0.564 (0.510–0.624)
426	5.0	7.6	6.57 (6.35–6.75)	7.76 (7.73–7.79)	12.5 (12.2–12.9)	39.1 (35.6–42.9)	0.610 (0.555–0.670)
420	4.6	7.3	5.66 (5.50–5.90)	7.75 (7.72–7.79)	12.7 (12.4–13.1)	32.1 (30.2–34.0)	0.497 (0.468–0.527)
414	3.2	6.5	5.47 (5.10–5.80)	7.75 (7.71–7.79)	13.0 (12.6–13.2)	40.6 —	0.643 —
398	1.7	5.6	4.40 (4.25–4.60)	7.76 (7.70–7.83)	12.5 (12.4–12.7)	33.1 (30.4–36.1)	0.517 (0.474–0.564)
405	2.3	6.2	4.37 (4.25–4.55)	7.78 (7.74–7.84)	12.4 (12.0–13.0)	33.4 (31.8–35.0)	0.541 (0.516–0.567)
450	9.4	9.6	3.56 (3.30–3.95)	7.87 (7.80–7.95)	12.6 (12.5–12.7)	23.9 (21.6–26.6)	0.482 (0.436–0.536)
452	10.1	9.7	3.21 (2.65–3.45)	7.79 (7.75–7.86)	12.6 (12.4–12.9)	23.7 —	0.399 —
464	8.8	9.1	2.65 (2.50–2.85)	7.92 (7.89–7.96)	12.8 (12.7–13.0)	14.4 (11.8–17.5)	0.330 (0.271–0.401)
457	8.2	8.7	2.64 (2.40–2.95)	7.89 (7.85–7.95)	12.5 (12.2–12.9)	15.1 (13.3–17.1)	0.316 (0.280–0.358)

[a]Averages and range of values in all tanks for all tests for other measured water chemistry variables (in milligrams per litre) were alkalinity (as $CaCO_3$), 172 (169–176); hardness (as $CaCO_3$), 205 (203–210); NO_2–N, 0.00 (0.00–0.01); and NO_3–N, 0.14 (0.06–0.19).

[b]Confidence intervals not calculable from statistical method used.

under constant pressure through flowmeters (Dwyer Instrument Corp.) at the rate of 100 mL/min. In these tests, toxicant solution was premixed into the dilution water at different rates for each tank by means of fixed-speed metering pumps (Barnant Corp.) and water replacement time in the tanks was ~10 h; full test toxicant concentration in each tank was effectively reached within 36 h.

Fish for a given test were selected at random 2–2.5 d prior to the start of the test, weighed collectively, and distributed (10 per tank) at random among the tanks. The D.O. concentration of the tank water into which the fish were placed was 8–9 mg/L. This water was then gradually replaced by water at the test D.O. concentration over the next 18–36 h; test D.O. concentration was effectively reached 0.5–1 d before introduction of toxicant. D.O. and flow rates for each tank were measured at least twice each day thereafter and flow rates were adjusted if needed. The lowest D.O. test completed was 2.6 mg/L, this being the lowest concentration at which 90% or more of the control fish survived. Measurements of fish for total length were taken as dead fish were removed from the tanks during each test or at the conclusion of each test.

Chemical analyses were performed on water in the test tanks using the following methods. Total ammonia–nitrogen (NH_3–N) was determined three or four times in each tank for each test (beginning on day 2) using the nesslerization method from APHA et al. (1976). Un-ionized ammonia (NH_3) concentrations were calculated from total ammonia concentrations in the individual test tanks using the formulas of Emerson et al. (1975) and the table of Thurston et al. (1979). Oxygen was measured using a Yellow Springs Instrument model 54 D.O. meter, temperature using a calibrated mercury thermometer, and pH with a Beckman Phasar-I digital meter. These measurements were taken each time total ammonia was measured. Average pH values were obtained by use of a computer program which converted each measurement to the hydrogen ion concentration, averaged these, then reconverted the average to pH units. Alkalinity, hardness, nitrate–nitrogen (NO_3–N), and nitrite–nitrogen (NO_2–N) were determined at least once in each tank for each test according to standard analytical procedures (APHA et al. 1976; U.S. EPA 1974). Colorimetric measurements were made using a Varian 635 ultraviolet–visible spectrophotometer.

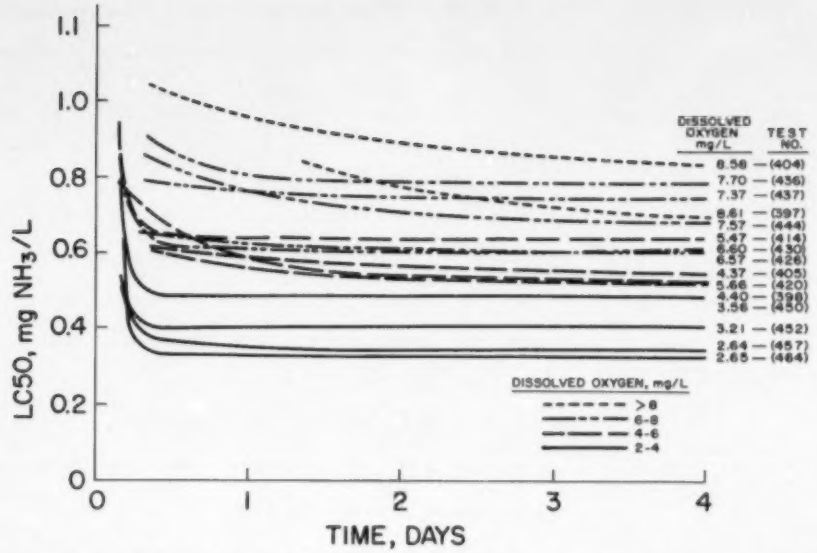

FIG. 1. Ammonia toxicity curves for 96-h bioassays on rainbow trout at different concentrations of dissolved oxygen.

Mortality observations were made at 4- to 8-h intervals during the first 24 h of each test and at 6- to 12-h intervals during the next 72 h. The median lethal concentrations (LC50) and their 95% confidence intervals were calculated for each test from the average NH_3–N and NH_3 values for each tank using the trimmed Spearman–Karber method (Hamilton et al. 1977). Toxicity curves were computer-generated using LC50 and time values for each time of mortality observation ($\geq$12 per test).

The 96-h LC50 (as milligrams NH_3 per litre) and D.O. (milligrams per litre) data were subjected to statistical correlation calculations. LC50 and ln (LC50) were both examined for possible correlations with D.O. concentration (including ln (D.O.) and $(D.O.)^2$). Because weight and length are always highly correlated (estimated correlation >0.95), and because weight is usually more highly correlated with LC50 than is length, we considered only weight as a possible covariable and calculated partial correlations for the 96-h LC50/D.O. data, holding fish weight constant. The correlations of D.O. vs. LC50 for the time periods of 12, 24, 48, 72, and 96 h were also compared to examine whether the effect of reduced oxygen concentration on ammonia toxicity was affected by duration of exposure. Finally, we examined our data to determine whether there was any evidence in our experiments of a greater effect of D.O. on survival time at low than at high concentrations of NH_3. For each test (each different D.O. concentration), the number of mortalities at 96 h for each of the five ammonia test tank concentrations was tabulated. The following linear relationship was assumed:

$$p = \beta_0 + \beta_1 \ln (NH_3) + \beta_2 \ln (D.O.) + \beta_{12} \ln (NH_3) \ln (D.O.)$$

where p = proportion mortality at 96 h, NH_3 = concentration of un-ionized ammonia in milligrams per litre, and D.O. = concentration of dissolved oxygen in milligrams per litre. The term $\beta_{12} \ln (NH_3) \ln (D.O.)$ is the interaction effect between NH_3 and D.O.

The hypothesis that D.O. concentration has a greater effect at low NH_3 concentration than at high can be restated as the hypothesis that β_{12}, the coefficient on the interaction term, is strictly positive; one expects β_2 to be negative. Therefore, the procedure used was to run a weighted linear regression of p on ln (NH_3), ln (D.O.), and the product ln (NH_3) ln (D.O.), test whether the coefficient β_{12} is significantly different from zero, and, if so, if it is positive. For completeness two other models to equalize variance were used (Snedecor and Cochran 1967); one of these involved arcsin transformation and the other logit transformation.

Results and discussion — Ninety-six-hour LC50 values (with 95% confidence intervals) under different conditions of D.O. are reported (Table 2) in terms of both NH_3–N and NH_3. Fish size and water measurements for D.O., pH, and temperature for each test are also reported (Table 2), as are summaries of the measured water chemistry variables considered not to have affected test results. There was one control mortality during one of the tests at the lowest D.O. level tested (test 464); there were no control mortalities in the other tests. Toxicity curves in terms of NH_3 are presented in Fig. 1. Most of the toxicity curves plateau by 4 d, giving asymptotic LC50 values ranging from 0.32 to 0.81 mg NH_3 per litre.

There was a strong positive correlation between D.O. (milligrams per litre) and LC50 (milligrams NH_3 per litre); as D.O. concentration decreased, NH_3 toxicity increased. The linear relationship between 96-h LC50 and D.O. concentration is illustrated in Fig. 2. The estimated correlation coefficient is 0.9346 (P = 0.00001; the estimated regression line

is LC50 = 0.1903 + 0.06712 (D.O.)). The highest estimated correlation of those calculated was between ln (LC50) and ln (D.O.) and equalled 0.9471 ($P = 0.00001$). The estimated partial correlation between ln (LC50) and ln (D.O.) holding weight fixed was 0.9064 ($P < 0.01$).

Analysis of the data to compare the D.O. vs. LC50 correlations at 12, 24, 48, 72, and 96 h showed a very clear trend (Fig. 3); the shorter the time period, the more pronounced the positive relationship between LC50 and D.O. concentration ($P < 0.0001$). This trend suggests at least two possibilities: either individual fish that require higher oxygen concentrations succumb early in the tests and/or those fish that do survive become increasingly acclimated to the ammonia and oxygen test conditions as time progresses.

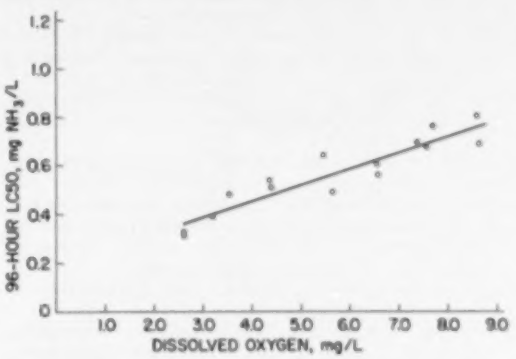

FIG. 2. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout: 96-h LC50 vs. D.O.

To test the hypothesis that the magnitude of the effect of reduced D.O. on ammonia toxicity is greater at low than at high concentrations of NH_3, we used both complete and truncated data sets. Analyzing the complete data set, using weighted linear regression, gives the following estimated model:

$$p = 1.86 + 0.734 \ln (NH_3) - 0.493 \ln (D.O.) + 0.031 \ln (NH_3) \ln (D.O.).$$

The estimated coefficient of the interaction term is then $\hat{\beta}_{12} = 0.031$. The coefficients on ln (NH_3) and ln (D.O.) are both significant (with P values of 0.000 and 0.002, respectively) but the coefficient for the interaction is not significantly different from zero ($P = 0.818$). Therefore, there is no evidence of any interaction between NH_3 concentration and D.O.

The truncated set consisted of only those treatments in which mortality was greater than 0% and less than 100%. The reasoning behind this truncation is that if no fish die at a low concentration of NH_3 coupled with a low level of D.O., no fish should die as the level of D.O. increases. Similarly, if all fish die at a high concentration of NH_3 coupled with a high level of D.O., there should still be 100% mortality as the level of D.O. decreases. When the truncated data set was used to run a weighted regression of p vs. ln (NH_3), ln (D.O.), and the product, all three coefficients were significant; there was a significant positive interaction ($P = 0.006$) and the hypothesis is accepted. This is a contradiction of the previous result. The analyses using arcsin transformation and logit transformation also yielded results that were not conclusive.

The discrepancy in results between using a complete data set and a truncated data set may be due to the inadequacy of

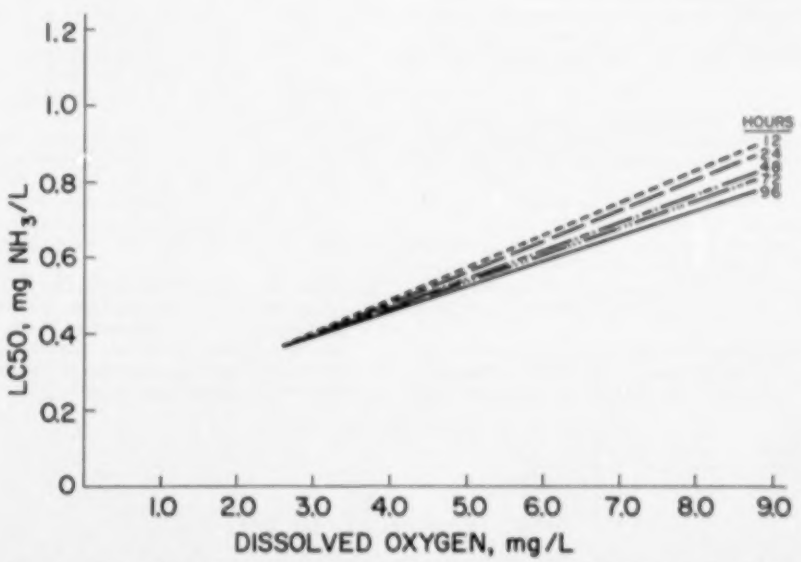

FIG. 3. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout: LC50 vs. D.O. at five time intervals.

the model to describe mortality behavior at the extreme values of p (0 or 1). The model may be more appropriate for the truncated data set, because this set was limited to those data covering the range wherein a change in concentration of D.O. and/or NH_3 had an effect on mortality. It would be unwise to draw any conclusions about interaction between NH_3 concentration and D.O. from only these data; future research should further address the question of interaction and a more complete model should be considered, one which might better handle the method of determining the region of D.O. and NH_3 values of interest.

Independent of conclusions from the preceding data analysis, the 96-h LC50 values are more often reached earlier in the tests at low D.O. concentrations than at high. This point is illustrated both by the 96-h toxicity curves (Fig. 1) and by the differences in slope of the regression lines (Fig. 3); the longer the time period, the less the slope.

The U.S. Environmental Protection Agency (1977) has recommended a minimum concentration of 5.0 mg D.O. per litre to maintain good freshwater fish populations. Our results show that any reduction in D.O., below the highest tested, reduced the tolerance of rainbow trout fingerlings to acutely toxic concentrations of ammonia; the estimated tolerance at 5.0 mg D.O. per litre is 30% less than that at 8.5 mg D.O. per litre. We recommend that water quality standards for D.O. in cold-water fish environments take into consideration background concentrations of ammonia which may be present and the likelihood of temporary increases in those concentrations.

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